Bull. Environm. Contam. Toxicol. 26, 508-512 (1981)

# Effect of Hexachlorocyclohexane on Somatic and Meiotic Divisions in Male Swiss Mice

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Hexachlorocyclohexane (HCH) is extensively used in India to control agricultural pests and vector-borne diseases such as malaria, thus increasing the risk of human exposure. Recently, NIGAM et al. (1979) reported testicular granuloma, hyalinization and degenerative changes in the testes of HCH-fed mice. However, reports on the effect of HCH on chromosomal preparations from bone marrow and testes are lacking in the literature. Therefore, in the present investigation an attempt has been made to pinpoint the effect of HCH on somatic and meiotic divisions and on chromosomes. Studies are further extended to elucidate whether the changes are permanent or reversible.

## MATERIALS AND METHODS

Male inbred Swiss mice of 6 to 8 weeks of age were used. Animals were fed a diet consisting of 70% cracked wheat, 20% cracked bengal gram, 5% fish meal, 4% yeast powder, 0.5% groundnut oil, and 0.5% shark liver oil in the form of dry mesh. In the experimental group, 500 ppm HCH was dissolved in alcohol and homogeneously mixed in the diet. Alcohol was allowed to evaporate. The technical grade HCH obtained from M/s Hindustan Insecticides Ltd., Delhi, India contained 13.5% of the gamma isomer. In group A animals were continuously fed HCH for 3, 5 and 8 mo and in group B, HCH was fed up to 5 and 8 mo followed by discontinuation of HCH for 2 months. Each batch consisted of six animals. At the termination of each batch of the experiment, equal number of animals of corresponding age group from control were taken.

Four h prior to sacrifice, animals were given an intraperitoneal injection of 0.2 mL of colchicine (0.01% aqueous). All the animals were sacrificed at about 3 p.m. The bone marrow was used for preparing chromosomes of somatic mitoses. Slides were made using the conventional air-drying technique of TJIO & WANG (1961) and stained with Giemsa (E. Merck) at pH 7.0 in Sorensen's buffer (1:50 dilution) for 10 min.

After colchicine treatment one testis of each animal was used for chromosome preparation and the other was immediately fixed in Bouin's fixative for histological studies. Chromosome preparations were made according to the method described by EVANS et al. (1964)

0007-4861/81/0026-0508 $01.20

and stained with Giemsa.

## RESULTS

Bone Marrow. One hundred metaphases were scanned from each experimental and control group for any possible chromosomal aberrations. Except for a telomeric association in one metaphase in the group fed HCH for 3 mo, a chromatin fragment in one metaphase in the group fed HCH for 8 mo, no significant chromosomal aberrations were observed. The mitotic index of treated groups also did not show any alteration when compared to controls. Thus, there was no observable effect of dietary HCH on chromosomes or mitoses.

Testes. Among various division stages present in testes, spermatogonial metaphase, diakinesis/metaphase I and metaphase II stages were considered to evaluate the effect of HCH. The cellular divisions in testes in 3 mo HCH-fed group apparently did not show any observable effect either on the frequency of divisions or on the structure of chromosomes. However, on further continuation of HCH feeding up to 5 and 8 mo (Group A), the spermatogonial and all the meitotic division stages showed a significant numerical reduction asserting the inhibitory effect of HCH. The quantitative assessment of the divisions was not possible in these groups due to the rarity of divisions.

In group B, where the HCH is discontinued for two mo, a significant recovery was observed and the number of divisions were comparable with corresponding control group. The degree of the recovery was the same in both the 5 and 8 mo groups, but the division figures from recovery groups showed an altered chromosome structure and morphology from those of controls (Fig. 1 a-c). Metaphases appeared starved with extreme reduction in chromosome size. This effect was best seen at spermatogonial divisions (Fig. 1 d). Some other abnormalities recorded were diffuse chromosomal structure and extensive stickiness (Fig. 1 e), thus sometimes obscuring the identity of the stage due to chromatin masses (Fig. 1 f). More than 200 division figures were enumerated to check the ratio of different divisions and incidence of polyploidy (Table 1). No significant alteration was observed in the ratio of division stages, but there was an increase in polyploidy in different divisions (Fig. 1 g-i) which was statistically significant at 5% level in diakinesis and metaphase I.

## DISCUSSION

In the present study for the first time the effect of continuous feeding of HCH at the 500 ppm level for 8 mo has been observed. It did not produce any observable effect on bone marrow divisions or chromosomes indicating non-clastogenic effect on bone marrow chromosomes. The lack of clastogenic effect of pesticides like endosulfan is sometimes attributed to its fast metabolism and quick elimination through excretion (DIKSHITH & DATTA 1978). On the other hand, DDT does not have direct clastogenic effect but its metabolites, e.g. DDE, show mutagenic effect (KELLY-GAVERT & LEGATOR 1973). However,

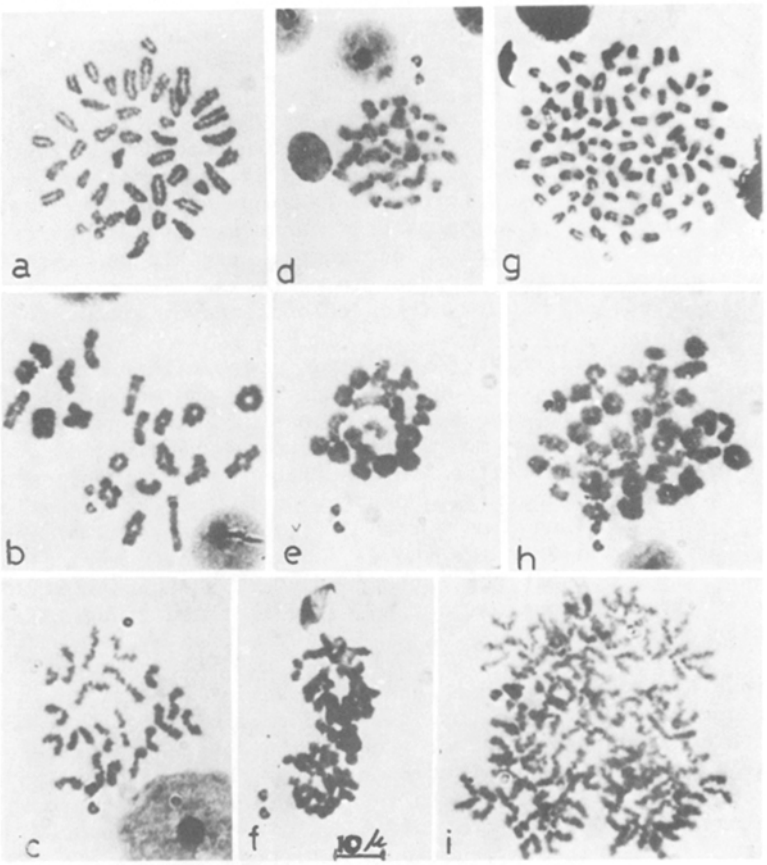


Figure 1:
a-c: Showing different division stages in testes of control mice;
d-i: Showing stages in testes of experimental mice. d, f and h are from 2 mo recovered group after 5 mo of 500 ppm HCH feeding; e, g and i are from 2 mo recovered group after 8 mo of 500 ppm HCH feeding.

Table 1. Cell Counts from Testes of Mice in Control and Recovering Mice for 2 mo after 5 and 8 mo of HCH-Feeding at 500 ppm in Diet

| Treatment | Total No. of cells | Spermato-gonial | | Diakinesis/ metaphase I | | Metaphase II | |
|---|---|---|---|---|---|---|---|
| | | 2n | >2n | 2n | >2n* | n | >n |
| Control | 237 | 24 | 1 | 89 | 1 | 110 | 12 |
| 5 months | 260 | 37 | 5 | 97 | 4 | 104 | 13 |
| 8 months | 246 | 24 | 3 | 100 | 10 | 93 | 16 |

* = Significant at 5%

in this investigation HCH is maintained continuously at the 500 ppm level in food and therefore, the levels of HCH and its metabolites are present in mice throughout the experiment but still no observable effect on somatic cell divisions or chromosomes of bone marrow was seen in the experimental group.

Contrary to these observations on somatic system, there are marked histological changes similar to those reported earlier by NIGAM et al. (1979) with significant inhibition of divisions in testicular tissue. The inhibitory effect is progressive in nature as the cell divisions are almost negligible at 5 mo and after. However, the reappearance of various divisions after HCH discontinuation clearly indicates that the deleterious effect of HCH on testis is not permanent. During recovery, all the divisions have reappeared in near normal ratio showing non-specific inhibition. However, the chromosome structure and morphology are far from the normal and the number of polyploid cells were considerably increased in recovering animals. The polyploid chromosomal complements observed in present study are possibly derived from multinucleated cells reported by NIGAM et al. (1979) which might have resulted from nuclear divisions and subsequent failure of cytokinesis. The other important abnormal phenomenon observed in testes due to HCH treatment is stickiness and diffuse morphology of chromosomes. MCGILL et al. (1974) have suggested that the chromosomal stickiness after treatment of certain clastogens may be due to abnormal condensation of chromatin so that fibrils of different chromosomes may get entangled with each other. But in the present study the parallel observation on bone marrow chromosomes showed that HCH does not have direct clastogenic effect. Hence, the chromosomal stickiness found in testes may not be an outcome of direct HCH effect on DNA or chromosomes but possibly due to altered physiology which interferes with normal condensation of chromatin. The studies on similar pesticide, like DDT, have shown that DDT induces cellular alterations in testes (KRAUSE et al. 1975) either through altered steroid metabolism in reproductive organs (SMITH et al. 1972) and/or by direct antigonadotrophic action (GELLERT et al. 1972). Therefore, it is presumed that HCH alters the physiological characteristics and more particularly the hormones essential for spermatogenesis with similar mechanism.

Regardless, the mechanisms involved in HCH causing stickiness, it would result in lagging of chromosomes during the movement of chromosome complements towards the poles leading either to unequal distribution of chromatin or loss of the same as micronuclei. If such resultant abnormal cells and polyploids undergo spermateliogenesis, they would give rise to abnormal sperms increasing the risk of abnormal embryos. Though the present investigation involves very high concentrations of HCH which may be rarely found in the environment, the potential danger of fertility and abnormal reproduction cannot be overlooked for workers in manufacturing and formulating plants and pesticide spraymen who are repeatedly exposed to this pesticide.

Acknowledgments. The technical assistance of K. Jain is gratefully acknowledged.

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